D97
ADE HPRT
RARE RE SITES
URA

PHASE Ic IgH

D132
TRP
17H
URA
LEU URA
HIS

PLATE ON SC—ADE—HIS
+CANAVANINE
+CYCLOHEXIMIDE
+5—FOA

SPORULATE

DIPLOID
Ade+His+Leu+Lys—Trp+Ura+
CanR CyhS
CanSCyhR

PICK WHITE (Ade+) COLONIES
TEST FOR Trp—Ura—Leu—

D97   D132   17H
ADE HPRT
RARE RE SITES
HIS

HAPLOID(α)
Ade+His+Leu—Lys—Trp—Ura—
CanR CyhR
Ⓧ
MATE

A287
TRP LYS
URA

AB1380(a)
Ade—His—Leu+Lys+Trp+Ura+
CanR CyhS

D97   D132   17H
ADE HPRT
RARE RE SITES
A287
TRP LYS
HIS

DIPLOID
Ade+His+Leu+Lys+Trp+Ura+
CanR CyhR
CanRCyhS

PLATE ON SC—ADE—URA
+CANAVANINE
+CYCLOHEXIMIDE

SPORULATE

PICK WHITE (Ade+) COLONIES
TEST FOR His— Trp—

FIG.1

D97   D132   17H   A287
ADE HPRT
RARE RE SITE
TARGET
URA
LYSNEO
Yₗ 3' ENHANCER
RARE RE SITE

D97   D132   17H   A287   Yₗ 3' ENHANCER
ADE HPRT
RARE RE SITE
LYSNEO
RARE RE SITE

FIG.2

RECOMBINATION OF YAC 203 WITH YAC 210:

203-C6 (450kb)

202/AD1360
α,ADE,LYS,HIS3,his5
ConR,Cyc5

MATE ON YPD PLATE

FIRST SELECTION OF DIPLOIDS ON-His PLATES
SECOND SELECTION ON -ura,-trp,-ado,lys

210/YAUS0
α,URA,TRP,his3,HIS5
ConR,Cyc5

A3/19 (II)

210-F10 (300kb):5-B V genes

PREPARATION OF DNA PLUGS
ANALYSIS ON CHEF
HYBRIDIZATION WITH A3 PROBE

2-FRAGMENTATION OF 203/210 WITH USING THE VA-A80L, FRAGMENT
LIGATED TO pYAC RIGHT ARM:

203/210 (4-8):800 kb

SELECTION ON SORB-URA ADE

203/210-A80L:600kb

Kde IS LOST AFTER THE FRAGMENTATION

mpy203/210(α)
ConR CyhS

MATE

DIPLOID
Ade+,Ura+,Lys+,Trp+
ConR CyhS
——————
ConS CyhR

YPH857 (α)
ConS CyhR

SPORULATE

y203/210

PLATE ON SC-Ade-Lys-Arg
+CANAVANINE + CYCLOHEXIMIDE
PICK WHITE (Ade+) COLONIES
TEST FOR Trp-,Ura-

(800 kb)

PLATE ON SC+5FOA
PICK WHITE (Ade+) COLONIES
TEST FOR Trp-,Ura-,Lys+Ade+

A80 (185 kb)

A80

OD 492nm

- □— hκ ANTI−IL6 REACTIVITY
- ○·· hμ ANTI−IL6 REACTIVITY
- ◇·· hκ ANTI−OVA neg. CONTROL
- △-- hμ ANTI−OVA neg. CONTROL

SERUM DILUTIONS

FIG.3

OD 492nm

- ○·· hκ ANTI−TNFα REACTIVITY
- □— hμ ANTI−TNFα REACTIVITY
- ◇·· hμ ANTI−IL8 neg. CONTROL
- △-- hκ ANTI−L−SELECT. neg. CONTROL

SERUM DILUTIONS

FIG.4

FIG.5

FIG.6

—□— hκ ANTI–3T3 L–SELECTION

--○-- hκ ANTI–3T3

OD 492nm

SERUM DILUTIONS

FIG.7

HUMAN NEUTROPHILS

XENOMOUSE
SERA 1:100

NEGATIVE CONTROL 1:100
ISOTYPE

M1

COUNTS

550
440
330
220
110
0

10^0 10^1 10^2 10^3 10^4
FL2-H

MOUSE ANTI-HUMAN KAPPA PE

FIG.8

h gp 39 cDNA

EMCV IRES

CMV PROMOTOR

176 NdeI
281 SnaBI
507 Ecl136I
507 SacI
595 SacII
593 NaII

1170 BclI
1278 BstEII

1557 XcmI
1612 PaeR7I
1612 XhoI

1940 PmlI
2067 Asp718
2067 KpnI
2073 PlMI
2348 BbeI
2348 EheI
2348 KasI
2348 NarI
2464 Tth111I
2864 RsrII

pIK1.1Hu gp39/IRES NEO copy

6825 BASE PAIRS
UNIQUE SITES

SpeI 6767

BsaI 5307

ScaI 4896

Amp R

puc 19

StuI 3777
SfiI 3726
HpaI 3503
3030 BstBI

FIG.9

OD 492nm

- ○---- hκ ANTI-CHO-gp39 REACTIVITY
- □—— hμ ANTI-CHO-gp39 REACTIVITY
- △--- hκ ANTI-PTHrp neg. CONTROL
- ◇···· hμ ANTI-TNFα neg. CONTROL

SERUM DILUTIONS

FIG.10

- □— D5.1/hμ
- ◪---- D5.1/hκ

DILUTIONS

FIG.11

```
                        CDR1
                    |        |
Germline   VH6      AGACCCTCTC ACTCACCTGT GCCATCTCCG GGACAGTGT CTCTAGCAAC  50
Hybridoma  D5. 1.4  AGACCCTCTC ACTCACCTGT GCCATCTCCG GGACAGTGT CTCTAGCGAC  50
Germline   JH4      ────────── ────────── ────────── ───────── ──────────
Germline   D(N1)    ────────── ────────── ────────── ───────── ──────────
Germline   hMu      ◄───────── ────────── ────────── ───────── VH6 ──────

                               CDR2
                       |                               |
Germline   VH6      AGTGCTGCTT GGAACTGGAT CAGGCAGTCC CCATCGAGAG GCCTTGAGTG  100
Hybridoma  D5. 1.4  AGTGCTGCTT GGAACTGGAT CAGGCAGTCC CCATCGAGAG GCCTTGAGTG  100
Germline   JH4      ────────── ────────── ────────── ────────── ──────────
Germline   D(N1)    ────────── ────────── ────────── ────────── ──────────
Germline   hMu      ────────── ────────── ────────── ────────── ──────────
                    ───────────────── VH6 ───────────────────────────────►

Germline   VH6      GCTGGGAAGG ACATACTACA GGTCCAAGTG GTATAATGAT TATGCAGTAT  150
Hybridoma  D5. 1.4  GCTGGGAAGG ACATACTACA GGTCCAAGTG GTATAATGAT TATGCAGTTT  150
Germline   JH4      ────────── ────────── ────────── ────────── ──────────
Germline   D(N1)    ────────── ────────── ────────── ────────── ──────────
Germline   hMu      ────────── ────────── ────────── ────────── ──────────
                    ─────────────── VH6 ─────────────────────────────────►

Germline   VH6      CTGTGAAAAG TCGAATAACC ATCAACCCAG ACACATCCAA GAACCAGTTC  200
Hybridoma  D5. 1.4  CTGTGAAAAG TCGAATAACC ATCAACCCAG ACACATCCAA GAACCAGTTC  200
Germline   JH4      ────────── ────────── ────────── ────────── ──────────
Germline   D(N1)    ────────── ────────── ────────── ────────── ──────────
Germline   hMu      ────────── ────────── ────────── ────────── ──────────
                    ─────────────── VH6 ─────────────────────────────────►

Germline   VH6      TCCCTGCAGC TGAACTCTGT GACTCCCGAG GACACGGCTG TGTATTACTG  250
Hybridoma  D5. 1.4  TCCCTGCAGC TGAACTCTGT GACTCCCGAG GACACGGCTG TGTATTACTG  250
Germline   JH4      ────────── ────────── ────────── ────────── ──────────
Germline   D(N1)    ────────── ────────── ────────── ────────── ──────────
Germline   hMu      ────────── ────────── ────────── ────────── ──────────
                    ─────────────── VH6 ─────────────────────────────────►

Germline   VH6      TGCAAGAGA─ ────────── ────────── ────────── ──────────  259
Hybridoma  D5. 1.4  TGCAAGAGAT ATAGCAGTGG CTGGCGTCCT CTTTGACTGC TGGGGCCAGG  300
Germline   JH4      ────────── ────────── ────────── CTTTGACTAGC TGGGGCCAAG  20
Germline   D(N1)    ─────────T ATAGCAGCAG CTGG────── ────────── ──────────  15
Germline   hMu      ────────── ────────── ────────── ────────── ──────────
                    ─── VH6 ───| ◄── DN1 ──| |◄── JH4 ───────
```

FIG.12A

```
Germline   VH6                  ————————  ————————  ————————  ————————  ———————— 259
Hybridoma  D5. 1.4   GAACCCTGGT CACCGTCTCC TCAGGGAGTG CATCCGCCCC AACCCTTTTC 350
Germline   JH4       GAACCCTGGT CACCGTCTCC TCA——————  ————————  ———————— 43
Germline   D(N1)                ————————  ————————  ————————  ————————  ———————— 15
Germline   hMu                  ————————  ————————  —GGGAGTG  CATCCGCCCC AACCCTTTTC 27
                     ————————— JH4 ——————————►|◄————— hμ ——————————
```

```
Germline   VH6                  ————————  ————————  ————————  ————————  ———————— 259
Hybridoma  D5. 1.4   CCCCTCGTCT CCTGTGAGAA TTCCCCGTCG GATACGAGCA GCGTGGCCGT 400
Germline   JH4                  ————————  ————————  ————————  ————————  ———————— 43
Germline   D(N1)                ————————  ————————  ————————  ————————  ———————— 15
Germline   hMu       CCCCTCGTCT CCTGTGAGAA TTCCCCGTCG GATACGAGCA GCGTGGCCGT 77
                     ————————————————————— hμ —————————————————————►
```

FIG.12B

```
Germline B3        GACATCGTGA TGACCCAGTC TCCAGACTCC CTGGCTGTGT CTCTGGGCGA
Hybridoma D5 1.4   ────────── ────────── ────────── ────────── ──────────
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────

                                          ┌──────────────── CDR1 ────────────────┐
Germline B3        GAGGGCCACC ATCAACTGCA AGTCCAGCCA GAGTGTTTTA TACAGCTCCA
Hybridoma D5 1.4   ─────ACC ATCAAGTGCA AGTCCAGCCA GAGTGTTTTG TACACTTCCA
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────
                        ◄──────────────────────── B3 ────────────────────────

Germline B3        ACAATAAGAA CTACTTAGCT TGGTACCAGC AGAAACCAGG ACAGCCTCCT
Hybridoma D5 1.4   GCAATAAGAA CTACTTAGCT TGGTACCAGC AGAAACCAGG ACAGCCTCCT
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────
                   ──────────────────────────── B3 ────────────────────────────►

                                     ┌──────────────── CDR2 ────────────────┐
Germline B3        AAGCTGCTCA TTTACTGGGC ATCTACCCGG GAATCCGGGG TCCCTGACCG
Hybridoma D5 1.4   AAACTACTCA TTTACTGGGC ATCTACCCGG GAATCCGGGG TCCCTGACCG
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────
                   ──────────────────────────── B3 ────────────────────────────►

Germline B3        ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC ATCAGCAGCC
Hybridoma D5 1.4   ATTCAGTGGC AGCGGGTCTG GGACAGATTT CACTCTCACC ATCGGCAGCC
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────
                   ──────────────────────────── B3 ────────────────────────────►

Germline B3        TGCAGGCTGA AGATGTGGCA GTTTATTACT GTCAGCAATA TTATAGTACT
Hybridoma D5 1.4   TGCAGGCTGA AGATGTGGCA GTTTATTACT GTCAGCAATA TTATACTATT
Germline JK3       ────────── ────────── ────────── ────────── ──────────
Germline CK        ────────── ────────── ────────── ────────── ──────────
                   ──────────────────────────── B3 ────────────────────────────►

Germline B3        CC──────── ────────── ────────── ────────── ──────────
Hybridoma D5 1.4   CCATTCAATT TCGGCCCTGG GACCAGAGTG GATATCAAAC GAACTGTGGC
Germline JK3       ─ATTCACTT TCGGCCCTGG GACCAAAGTG GATATCAAAC ──────────
Germline CK        ────────── ────────── ────────── ────────── GAACTGTGGC
                   ◄──┤◄──────────────────── JK3 ────────────────►├◄─CK─
```

FIG.13A

Germline B3 ———————— ———————— ———————— ———————— ————————
Hybridoma D5 1.4 TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG TTGAAATCTG
Germline JK3 ———————— ———————— ———————— ———————— ————————
Germline CK TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG TTGAAATCTG
———————————————————————— CK ————————————————————————▶

Germline B3 ———————— ———————— ———————— ———————— ————————
Hybridoma D5 1.4 GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC
Germline JK3 ———————— ———————— ———————— ———————— ————————
Germline CK GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC
———————————————————————— CK ————————————————————————▶

Germline B3 ———————— ———————— ———————— ———————— ————————
Hybridoma D5 1.4 AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTT GGGGAAAAA
Germline JK3 ———————— ———————— ———————— ———————— ————————
Germline CK AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGT— ————————
———————————————————————— CK ————————————————————————▶

FIG.13B

APPROVED BY DRAFTSMAN
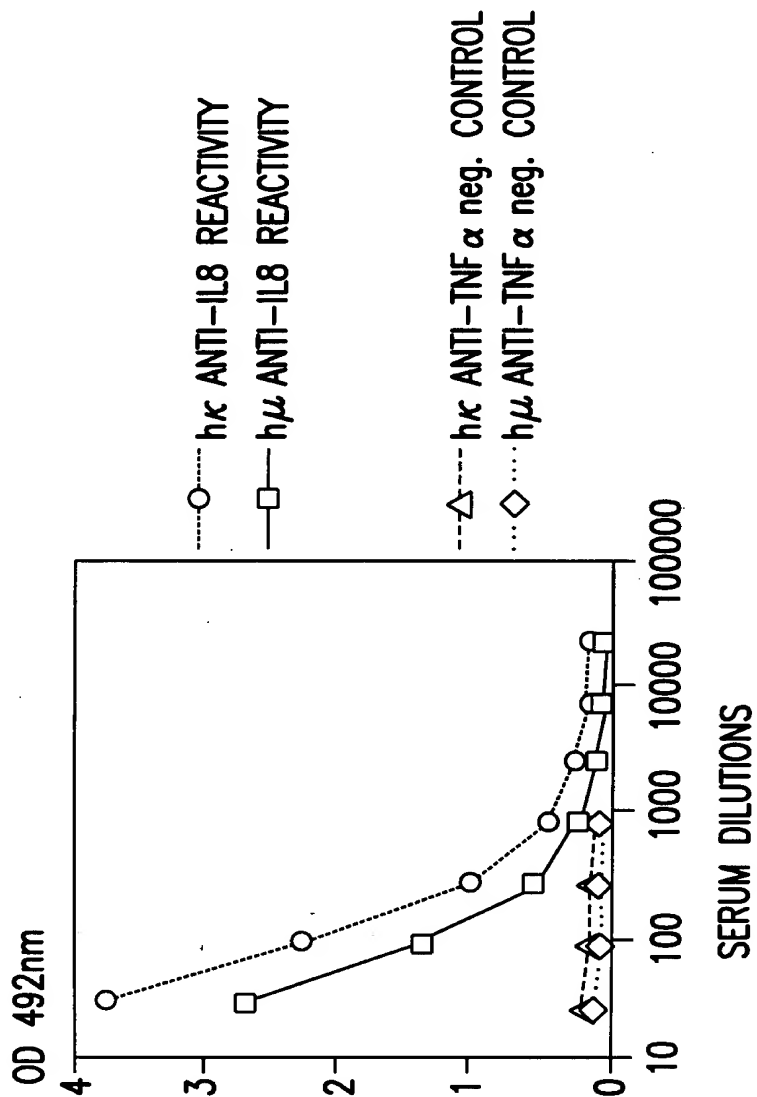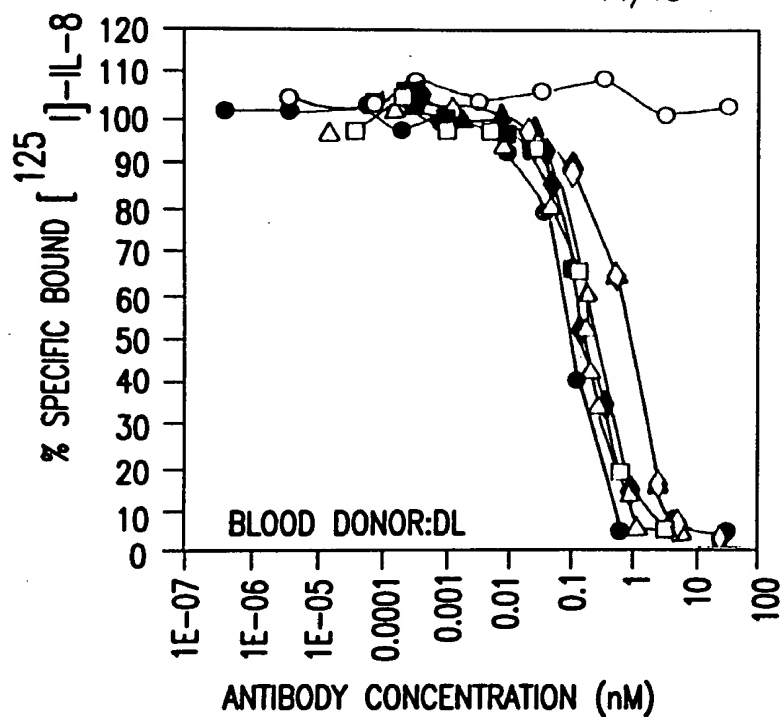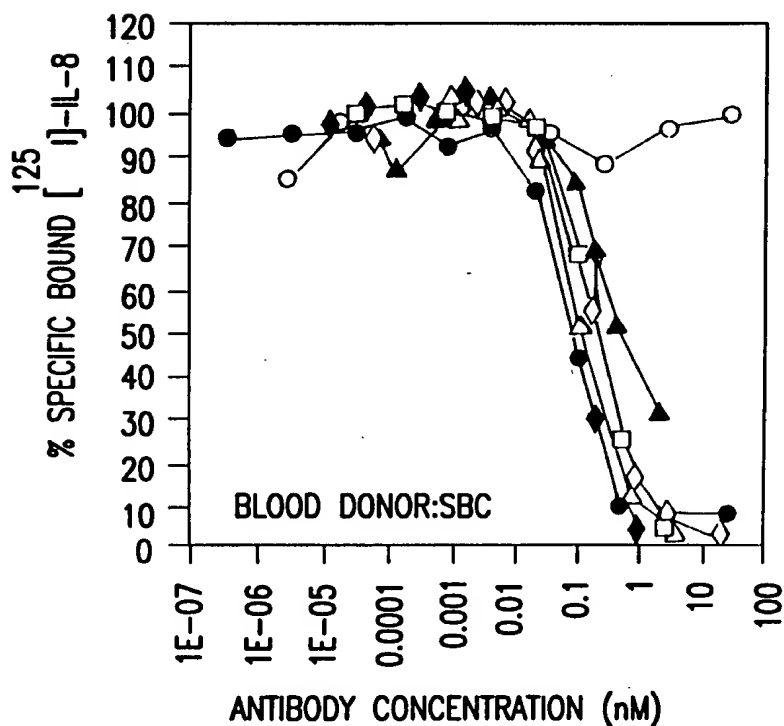
O.G. FIG.

CLASS | SUBCLASS

OD 492nm

SERUM DILUTIONS

hκ ANTI-IL8 REACTIVITY

hμ ANTI-IL8 REACTIVITY

hκ ANTI-TNFα neg. CONTROL

hμ ANTI-TNFα neg. CONTROL

FIG.14

FIG.15A

FIG.15B

[CCTGTCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCC

AGCCCCCAGGGAAGGGACTGGAGTGGATTGGGGAAATCAATCAAAGTGGAAGCACCAATTACAA

CCCGTCCCTCAAGAGTCGAGTCATCATATCAATAGACACGTCCAAGACCCAGTTCTCCCTGAAGT

TGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGA][GACTCCCC][ATGCT

TTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCTTCAG]CCTCCACCAAGGGCCCATCGG

TCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GCCCTGGGCTGCCTG

GTCAAGGACTACTTCC

FIG. 16A

[CAGTCTCCATCCTCCCTGTCTGCATCTGTAGGCGACAGAGTCACCATCACTTGCCAGGCGAGTC

AGGACATTAGTAAGTTTTTAAGTTGGTTTCAACAGAAACCAGGGAAAGCCCCTAAACTCCTGATC

TACGGTACATCCTATTTGGAAACCGGGGTCCCATCAAGTTTCAGTGGAAGTGGATCTGGGACAGA

TTTTACTCTCACCATCAGCAGCCTGCAGCCTGAAGATGTTGCAACATATTTCTGTAACAGNATG

ATGATCTCCC][ATACACTTTCGGCCCTGGGACCAAAGTGGATATCAAAC]GAACTGTGGCTGCAC

CATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCC

TGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC

FIG. 16B

[AGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGNT

CCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGAAATATCATATGATGGAAGTAATAAA

TACTATGTAGACTCCGTGAAGGGCCGACTCACCATCTCCAGAGACAATTCCAAGAACACGCTGT

ATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGA][CCGAC

TGGGGAT][CTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGG

GCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCT

GGGCTGCCTGGTCCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTC

TGACCAG

FIG. 16C

[CTGACNCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAGGGCCACCATCAACTGCAAGT

CCAGCCAGAGTGTTTTATACATCTCCAACAATAAAACTACTTAGCTTGGTACCAGCAGAAACCA

GGACAGTCTCCTAAACTGCTCATTTACTGGGCATCTACCCGGAAATCCGGGGTCCCTGACCGATT

CAGTGGCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAGATGTG

GCAGTTTATTACTGTCAACAGTATTATGATACTCC][ATTCACTTTCGGCCCTGGGACCAAAGTGG

ATATCAAAC]GAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAA

TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG

GAAGGTGGNTAACGCCCCA

FIG. 16D

[TCCCTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGACCTGGATCCGCCAGCC

CCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCATTCATCATGGAAACACCAACTACAACCCG

TCCCTCAAGAGTCGAGTCTCCATATCAGTTGACACGTCCAAGAACCAGTTCTCCCTGACACTGAG

CTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGAGG][GGGAGCAGTGGCTGCG][T

TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGGGCCCATCGGT

CTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCTGGGCTGCCTG

GTCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCTGACCAGCGGC

GTGCACACCTTCCCA

FIG. 16E

[TGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAGGC

GAGTCAGGACATTAGTAACTATTTAAATTGGTATCAACAGAAAGCAGGGAAAGCCCCTAAGGTCC

TGATCTACGCTGCATCCAATTTGGAAGCAGGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGG

ACAGATTTTACTTTCACCATCAGCAGCCTGCAGCCTGAAGATATTGCAACATATTATTGTCAACA

CTATGATAATCT]A[CTCACTTTCGGCGGAGGGACCAAGGTAGAGATCAAAC]GAACTGTGGCTGC

ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGACTGCCTCTGTTGTGTG

CCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG

FIG. 16F

AGTCTCTGAAGATCTCCTGTAAGGGGTTCTGGATACAGCTTTACCAGCTACTGGATCGGCTGGGTG

CGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGGATCATCTATCCTGGTGACTCTGATACCA

GATACAGCCCGTCCTTCCAAGGCCAGGTCACCATCTCAGCCGACAAGTCCATCAGCACCGCCTA

CCTGCAGTGGAGCAGCCTGAAGGCCTCGGACACCGCCATGTATTACTGTGCGAGACA][GGACGG

TG][ACTCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAG]CCTCCACCAAGGG

CCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGC(GC)GGCCCTG

GGCTGCCTGGTCCAAGGACTACTTCCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCTCT

GACCAGCGGCGTGCACACCTTCCCACTGCCA

FIG. 16G

TGTCTGCATCTATTGGAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAACTA

TTTAAATTGGTATCAGCAGAAACCAGGGCAAAGCCCCTAAGTTCCTGATCTATGGTGCATCCAGT

TTGGAAAGTGGGGTCCCATCANGGTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCAT

CAGCAGCCTGCAACCTGNGGATTTTGCAACTTACTACTGTCAACAGAGTTACAGTAACCC]T[CTC

ACTTTCGGCGGNGGGACCAANGTGGAGATCAAAC]GAACTGTGGCTGCACCATCTGTCTTCATCT

TCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCT

ATCCCAGAGAGGCCAAAGTACA

FIG. 16H